

Potassium and Apoptosis

Scientists in the Laboratory of Signal Transduction at the NIEHS have broken new ground in the study of the effect of ions, particularly potassium (K^+), on apoptosis, or "programmed cell death." It is known that apoptosis plays an important role in the development of disease, but little is understood about how this mechanism works. In two papers soon to be published in the *Journal of Biological Chemistry*, investigators John Cidlowski, Carl Bortner, and Francis Hughes, Jr. explain that the loss of K^+ from a cell is a necessary precursor to apoptosis. This is a reversal in the understanding of the role of ion flux in apoptosis; it was thought previously that cell-size modulation and ion flux are secondary events in apoptosis. But these new findings indicate that ion efflux is actually a very early event in cell death.

All cells contain the genetically coded mechanism to begin the apoptotic process, a sort of cell "suicide." Several things happen to a cell during apoptosis. The most obvious and characteristic change is that the cell shrinks. Inside the shrunken cell, the activity levels of certain enzymes escalate, leading to a breakdown of the cell's DNA structure. At this point, the cell body begins to fragment, whereupon it is engulfed by surrounding phagocytic cells. The cascade of events leading to eventual cell death by apoptosis may be triggered by a number of chemical signals. For example, death may occur when a cell is no longer needed, such as when a human fetus loses the webbing between its fingers or when the endometrial lining of the uterus is sloughed off during menstruation, or when

DNA-level damage is detected in a cell, as may occur following infection with a virus.

Because apoptosis is an organism's way of maintaining healthy cell populations, the process, if unduly suppressed or stimulated, can lead to disease; in fact, the phenomenon of apoptosis is a key factor in the development of almost all diseases. For example, in such neurodegenerative diseases as Alzheimer's, heightened apoptosis leads to the premature and exaggerated loss of cells. Conversely, cancer may be the result of a failure in the apoptotic process, in which mutant cells are allowed to proliferate freely rather than being recognized as damaged and destroyed. It's believed that a process that plays such a central role in the development of disease could also play a central role in the prevention of disease. This is the concept behind chemotherapy, which triggers apoptosis by damaging cells, thereby causing the cancerous cells (albeit along with many healthy cells) to die. But chemotherapy and other such treatments don't always work because scientists haven't been sure how to control the apoptotic process. This may be about to change. In their *JBC* papers, the NIEHS scientists demonstrate that the loss of K^+ from a cell is an important signal requirement for apoptosis to begin, rather than a side-effect of the process, as was previously believed.

Much research into apoptosis has centered on the role that enzymes and tumor suppressor genes such as *p53* are thought to play. But because cell shrinkage is routinely regulated by ions and because enzyme activity is often influenced by ion levels, the

NIEHS investigators focused their examination on the effect of ionic changes on the activity of a cell's programmed apoptotic machinery. Specifically, they studied the effect of K^+ in relation to two of the enzymes whose activity level rises in the apoptotic cell: the internucleosomal DNA cleavage nuclease and caspase-3-like protease.

The relationship between cell shrinkage and fragmentation was studied using a combination of flow cytometry, inductively coupled plasma/mass spectrometry, and a fluorometric assay. While K^+ is the primary ion found inside a cell, the cell's external environment is rich in sodium; a constant cell size is maintained as long as the internal and external concentrations of these two ions remain fixed in relation to one another. But in apoptosis, the researchers found, K^+ flows steadily out of the cell, causing a loss in cell volume. And this shrinking of the cell appears to be closely linked to the activation of the enzymes that spur the degradation of the cell's DNA structure, resulting in cell death.

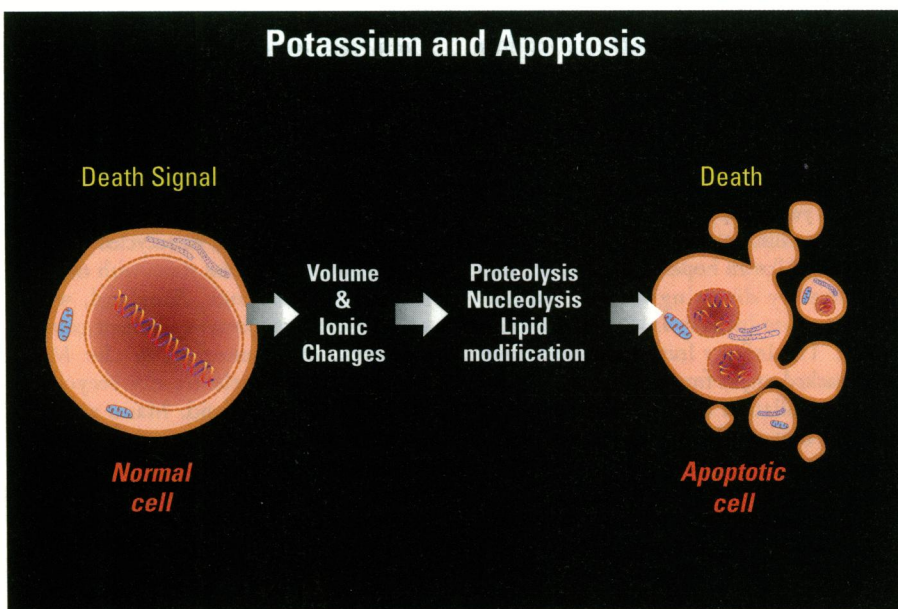
The discovery that K^+ efflux occurs very early in the process sheds light on the order of the chain of events involved in apoptosis, giving researchers a new way to look at how this process might be manipulated. Once a mechanism for voluntarily inducing or suppressing apoptosis is identified, a powerful tool will have been added to the arsenal against disease.

Well, Well, Well Water

A report recently issued by the General Accounting Office (GAO) suggests that many of the nation's private domestic wells are contaminated with excessive levels of nitrate and coliform bacteria. Entitled *Information on the Quality of Water Found at Community Water Systems and Private Wells*, the June 1997 report presents the results of an extensive investigation of private wells and community water supplies in the states of California, Illinois, Nebraska, New Hampshire, North Carolina, and Wisconsin.

According to Luther Atkins, the assistant director of the team that prepared the report in the resources, community, and economic development division at the GAO, the six states were selected based upon the amount of available data on private wells and the percentage of households that use well water for drinking purposes. "There's no single repository of data that speaks to contamination in private wells," Atkins says. "We had to depend on the states and other available studies . . . [and] we tried to get good geographical representation." Most of the data found by the GAO researchers were for total coliform bacteria and nitrate. The limited

Potassium and Apoptosis



K^+ amikaze cells. NIEHS researchers have uncovered another clue in the mystery of apoptosis—the release of potassium (K^+) from a cell may cause cell shrinkage and spur the degradation of a cell's DNA, leading to cell death.

data that were available for chemical pollutants such as pesticides, metals, and volatile organic compounds indicated that contamination with these agents is rare, with only 1–2% of wells tested reporting concentrations above federal maximum contaminant levels (MCLs).

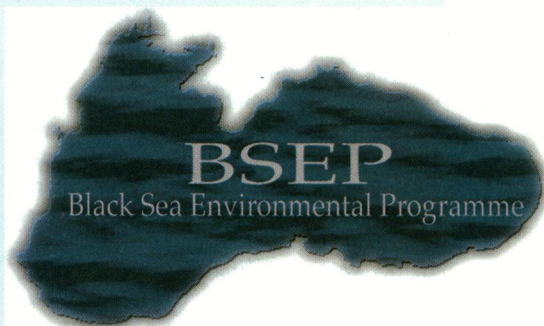
According to the GAO report, approximately 15 million households in the United States get their drinking water from private wells. Unlike community water supplies, which are regulated under the Safe Drinking Water Act (SDWA), there is no legislative mandate to monitor private wells on a regular basis. A number of states do require testing when new wells are installed, and testing of well water is a standard practice during property transactions. However, aside from these initial evaluations, routine testing is up to the homeowner's discretion. Robert J. Blanco, director of the implementation and assistance division in the Office of Groundwater and Drinking Water at the EPA and a principle reviewer of the report, says he wasn't surprised at the GAO's finding that up to 42% of private wells were contaminated with coliform bacteria at levels in excess of the MCL (as opposed to 3–6% of community systems), and that up to 18% exceeded the MCL for nitrate. "Contamination of private wells is common," he says. "As a former user of private well water, I would [recommend] testing well water periodically to make sure it's free of chemical and microbial pollutants."

Atkins emphasizes that the presence of coliform bacteria in and of itself does not necessarily mean that the water is unsafe to drink. However, he cautions, excessive concentrations of coliform bacteria—a general category of water quality indicators—may indicate the presence of more virulent microbes such as *E. coli*, and homeowners who detect elevated levels of coliforms in their water should test for these agents as well. Exposure to elevated levels of nitrate can have potentially serious consequences, particularly among infants who may respond by developing methemoglobinemia, or "blue baby syndrome," a potentially fatal condition that results from restricted transport of oxygen in the blood. The report didn't evaluate whether specific findings of elevated contaminant levels in well water were linked with increased incidence of health effects. Atkins says that such a link would have been difficult to assess because the kinds of health effects most often associated with contaminated water—such as acute gastrointestinal distress following exposure to bacterial agents—are typically not reported to public health agencies unless there is a community-wide outbreak, caused perhaps by contamination of a municipal system.

EHPnet

Black Sea Change

Perhaps it is appropriate that International Black Sea Day is recognized on October 31, the same day that many people observe Halloween. After all, not only is this the sea into which the rivers of the legendary Transylvania flow, it is also the scene of an environmental nightmare. Untreated wastes from countries such as Turkey, Ukraine, and the Russian Federation have accumulated and devastated fish populations in the Black Sea. Here, an exotic jellyfish population, accidentally released along with a ship's ballast water, has thrived, killing native fauna and reaching a total biomass of over 900 million tons, or ten times the weight of the world's annual fish catch. In short, the Black Sea is one of the most polluted bodies of water in the world.



However, because of International Black Sea Day and the organization behind it, conditions in this important European body of water may finally be improving. The Black Sea Environment Programme (BSEP) was formed in 1993 by the nations that border the sea with funding from the Global Environment Facility, a consortium of the United Nations Environment and Development programs and the World Bank. Inspired by the international cooperation displayed at the 1992 Rio Earth Summit, lawmakers from the governments of the six countries that border the Black Sea reached the conclusion that only a concerted effort could stop the sea's death.

As many of these nations were in the midst of profound social changes and lacked modern means of communication at the time the BSEP was formed, bringing them together to tackle the problems of the Black Sea presented a particular problem. To overcome these obstacles, the BSEP established an activity center in each of the six nations, each dedicated to researching one aspect of the Black Sea's environment, and linked the centers via an electronic mail network. To communicate the needs and achievements of the BSEP to the rest of the world, the program created the Save the Black Sea Internet site at <http://www.domi.invenis.com.tr/blacksea/index.htm>.

For scientists, governments, and nongovernmental organizations (NGOs) who want to help this heavily polluted region recover, the site provides information for contacting scientists in each nation and in the supporting organizations. Thus far, the program has drawn additional support from the governments of the Netherlands, France, Austria, Canada, and Japan, and recently 39 new NGOs also joined the effort.

The site provides extensive details to these donors about the activities of the BSEP and, in doing so, provides a model to the rest of the world of how environmental protection can be achieved, even in the midst of social upheaval. Recent successes of the program, including establishing an infrastructure for pollution monitoring, fishery management, and emergency response, are described through links on the home page. One of the biggest successes of the program, described under the news link at the top of the home page, was the 1996 signing of the Black Sea Strategic Action Plan, in which the Black Sea nations pledged themselves to a comprehensive plan to reverse the environmental damage that has been done. The 31 October 1997 anniversary of this historical agreement was declared International Black Sea Day, and if the program is successful, that day should become anything but scary for the people who live in the nations that share this deteriorated body of water.

GAO investigators found that a number of factors influenced the quality of water in both community systems and private wells. Foremost among these is the quality of the water at its source; water that is contaminated at its source will also be contaminated at the tap if it has not undergone sufficient treatment. Other factors include the degree to which construction standards are adhered, and the extent of ongoing maintenance and oversight of water supplies.

The final recommendation of the report is that the EPA should explore options for informing private well water users on how to maintain optimal quality of their drinking water. Atkins comments that the best warning scenario for homeowners with private wells that draw off the same aquifers as community systems is through the local media, which can alert them to contamination of the municipal supply. "There's already a system in place to alert the public of contamination in the community supply under [the] SDWA," he says. "Therefore, the GAO recommended that the EPA explore options to also alert private water users to consider testing their water whenever a groundwater-based community system detects contamination that could also be present in nearby private wells."

Industrial Chemical Mixture

The Chemical Industry Institute of Toxicology (CIIT) of Research Triangle Park, North Carolina, has announced a new research partnership with the Arlington, Virginia-based Chemical Manufacturers Association (CMA), a national trade association that represents over 180 major chemical producers. The alliance, established on 14 May 1997, is intended to extend the amount of research that the CIIT is able to perform, with the goal of providing an increasingly solid basis for chemical risk assessment.

The partnership will fund a state-of-the-science review that, according to CMA president Frederick L. Webber, will "identify emerging issues and determine the current scientific knowledge base as well as the gaps and uncertainties in our knowledge base." The CMA plans to provide \$20–25 million for research conducted at the CIIT and through external research grants. The initial commitment is a two-year pledge of \$16.5 million to support ongoing research at the CIIT, extramural research, and new initiatives resulting from the state-of-the-science review.

The state-of-the-science review will draw on the expertise of approximately 100 scientists from the CIIT, government, industry, and academia, whose efforts will be coordinated by a review committee. The review

committee has already compiled a list of nine high-priority research issues: the characterization of low-dose effects, risk assessment methodologies, sensitive subpopulations, ecosystem health, the relationship between biomarkers of exposure and effect, endocrine disruption, exposure assessment, neurobehavioral toxicology, and volatile organic compound reactivity in the atmosphere. The next step is to develop nine "white papers," which will examine each of these issues in the context of nine different disciplines, including carcinogenicity, developmental and endocrine sciences, neurotoxicity, respiratory toxicity, immunotoxicity, atmospheric chemistry, exposure assessment, risk assessment methodologies, and environmental toxicity. This process will illuminate exactly where gaps appear within the body of toxicologic knowledge and, therefore, where the partnership's research interests lie. "The continuing challenge for the industry," said Gary Van Gelder, manager of toxicology for Shell Chemical Company in Houston, Texas, and chairman of the review committee, "is to prioritize and target its research on issues where an incremental amount of new knowledge will improve public and environmental health in areas where the chemical industry has some involvement."

The CIIT is an independent entity and, as such, some have questioned the propriety of its funding coming from an advocacy group such as the CMA. But in a speech to CIIT board members at the institute's annual meeting last May, Webber explained that the CMA and the CIIT have common goals, including a commitment to reducing the uncertainties that currently confound scientists' ability to assess human risk from chemical exposures. In his speech Webber said, "[W]e believe that the institute can serve as an honest broker of knowledge on chemical health effects. Credibility and integrity are the institute's most prized possessions. . . . The chemical industry will lose—and lose big—if the institute's credibility is compromised in any fashion. We cannot afford to let that happen."

In his speech, Webber also delineated the partnership's four governing principles, which he called "a fire wall to protect the independence and integrity of the institute's work." First, all research will be conducted according to the most rigorous scientific principles and laboratory practices. Second, the partnership's board of directors, composed mostly of executives from CMA member companies, will help develop the research agenda and will provide the funds, but will play no role in directing the performance of the research. Third, all research will be made public in a timely manner—preferably in a peer-reviewed journal—regardless of whether

the expected impact of the results on the chemical industry is negative or positive. And fourth, a science advisory committee consisting of government and academic experts will oversee all phases of the research initiative, review all research activities, and maintain a check-and-balance on the quality of the research conducted.

The CIIT is a nonprofit research facility that was established in 1974 with the mission of strengthening and expanding the body of scientific knowledge on the potential human health effects of chemicals, pharmaceuticals, and other consumer products. The institute receives dues-based funding from approximately 36 private companies and trade associations, and maintains collaborative agreements with the NIEHS, the NTP, and the EPA. Over its 23 years of carrying out scientific studies, the CIIT has emerged as a respected leader in toxicological research.

While the CMA is known primarily as an advocacy group, it has also demonstrated an organizational commitment to environmental and social responsibility. One of the group's main achievements in recent years was the development of the Responsible Care program. Designed to improve health, safety, and environmental performance in the chemical industry, the program incorporates 106 different codes of practice into a comprehensive industry-wide management system. All CMA member companies are required to endorse and fully participate in the Responsible Care program.

Erratum

A table in the Forum of the October 1997 issue entitled "Summary of Substances, Mixtures, or Exposure Circumstances Nominated for Consideration of Listing in or Delisting from the Ninth *Report on Carcinogens*" incorrectly listed the status of several substances. Tobacco smoke, tamoxifen, UV radiation, and sulfuric acid mists were nominated to be listed as "Known to Be a Human Carcinogen," not "Reasonably Anticipated to Be a Human Carcinogen" as the table indicated. In addition, at its October 31 meeting, the NTP Board of Scientific Counselors' *Report on Carcinogens* Subcommittee did not recommend that saccharin be delisted from the *Report on Carcinogens*. The board's recommendations are the first of three reviews of the proposed actions.